

Serial No.: 09/954,737  
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#### Amendments to the Specification

Please replace the Sequence Listing on pages 1-9 as filed on June 4, 2004, with a substitute Sequence Listing presented on pages 1-24, enclosed herewith.

On page 5, please replace the paragraph starting on line 4 with the following:

Fig. 1A-B. **Tat components of *B. subtilis* and *E. coli*.** The amino acid sequences of Tat components of *B. subtilis* and *E. coli* as deduced from the SubtiList (<http://bioweb.pasteur.fr/Genolist/SubtiList.html>) and Colibri (<http://bioweb.pasteur.fr/Genolist/Colibri.html>) databases were used for comparisons. Identical amino acids, or conservative replacements are marked. Putative transmembrane segments, indicated in gray shading, were predicted with the TopPred2 algorithm (34, 35) (A) Comparison of TatAc (YnzA, SEQ ID NO:5), TatAd (YczB, SEQ ID NO:4) and TatAy (YdiI, SEQ ID NO:3) of *B. subtilis* (Bsu) with TatA (SEQ ID NO:1), TatB (SEQ ID NO:6) and TatE of *E. coli* (Eco) (SEQ ID NO: 24-6). (B) Comparison of TatCd (YcbT, SEQ ID NO:9) and TatCy (YdiJ, SEQ ID NO:8) of *B. subtilis* with TatC of *E. coli* (SEQ ID NO: 7[[[-9]]]).

On page 7, please replace the paragraph starting on line 14 with the following:

Fig 7. **Predicted twin-arginine (RR-)signal peptides of *B. subtilis*.** The listed signal peptides contain, in addition to the twin-arginines, at least one other residue of the consensus sequence (R-R-X-ΦΦ; printed in bold). The number of residues in the N- and H-domains of each signal peptide, and the average hydrophobicity (h) of each of these domains, as determined by the algorithms of Kyte and Doolittle (Kyte, J., and R. F. Doolittle [1982] A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 157:105-32), are indicated. Furthermore, the RR-motifs in the N-domain, and SPase I recognition sites in the C-domain (ie. positions -3 to -1 relative to the predicted SPase cleavage site) are shown. Proteins lacking a (putative) SPase I cleavage site, some of which contain additional transmembrane domains, are indicated with "TM". One protein containing cell wall binding repeats is indicated with "W".

Please replace Table I and the text following the Table, on page 56 with the following:

**Table I.** Predicted Twin-Arginine Signal Peptides of *B. subtilis*\*

protein	signal peptide	SEQ ID NO
AlbB	MSPAQRRLLYILSFIVIGAVVYFVKSDYLFTLIFIAIALF	84
AmyX <sup>T</sup> <sub>M</sub>	MVSIRRSFEAYVDDMNIITVLIPAEQKEIM	53
AppB <sup>TM</sup>	MAAYIIRRTLMSIPILLGITLSFVIMKAAPG	54
LipA	MKFVKRRRIALVTILMLSVTSLFALQPSAKAAEH	55
OppB <sup>TM</sup>	MLKYIGRRLVYMIITLFVIVTVTFFLMQAAPG	56
PbpX	MTSPTRRRRTAKRRRRKLNKRGKLLFGLLAVMVCITIWNALHR	57
PhoD	MAYDSRFDEWVQKLKEESFQNNTFDRRKFIQGAGKIAGLSLGLTIAQSVG AFEV	58
QcrA	MGGKHDISRRQFLNYTLTGVGGFMAASMLMPMVRFA	59
SpolIJ	MLLKRRIGLLLSMVGVMFLLAGCSSV	60
TipA <sup>TM</sup>	MKKTLTITIRRSSIARRLIISFLLILVPIITALSVSAYQS	61
WapA	MKKRKRRNFKRFAAFLVLALMISLVPADVLAKST	62
WprA	MKRRKFSSVVA AVLIFALIFSLFSPGTKAAAAGA	85
YeeA <sup>TM</sup>	MEMFDLEFMRRRAFLAGGMIAVMAPI LGVYLVLRRQ	64
YdeJ	MKKRRKICYCNTALLMILLAGCTDS	65
YdhF	MRRILSILVFAIMLAGCSSN	66
YdhK	MSAGKSYRKKMKQRRMNMKISKYALGILMLSLVFVLSACGNNN	67
YesM <sup>TM</sup>	MKKRVAGWYRRMKIKDKLFVFLSLIMAVSFLFVYSGVQYAFHV	86
YesW	MRRSCLMIRRRKRMFTA VTLVLVLMGTSVCPVKAEGA	69
YfkN <sup>TM</sup>	MRIQKRRTHTVENILRILLPPIMILSLILPTPIHAEES	70
YkpC	MLRDLGRRVVAIAAILSGIILGGMSISLANMP	71
YkuE	MKKMSRRQFLKGMFGALAAGALTAGGGYGYARYL	72
YmaC	MRRFLLNVILVLAIVLFLRYVHYSLEPE	73
YmzC	MFESEAE LRRIRIALVWIAVFLF GACGN	74
YubF <sup>TM</sup>	MQKYRRRNTVAFTVLAYFTFFAGVFLFSIGLYNADNL	75
YuiC <sup>TM</sup>	MMLNMIRLLMTCLFLLAFGTTFLSVSGIEAKDL	76
YvhJ	MAERVVRVRVKKKKSKRRKILKRIMLLFALALLVVGLGGYKLY	77
YwbN	MSDEQKKPEQIHRRDILKWGAMAGAAVAIGASGLGGLAPLVQTAAKP	78

\*Putative twin-arginine signal peptides were identified in two ways. First, the presence of the consensus sequence R-R-X-Φ-Φ (Φ is a hydrophobic residue), immediately in front of an amino-terminal hydrophobic region as predicted with the TopPred2 algorithm (34, 35), was determined. To this purpose, the first 60 residues of all annotated proteins of *B. subtilis* in the SubtiList database (<http://bioweb.pasteur.fr/Genolist/Subtilist.html>) were used. Second, within the group of twin-arginine membrane sorting signals, cleavable signal peptides were identified

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with the SignalP algorithm (61, 62). Conserved residues of the twin-arginine consensus sequence R-R-X-Φ-Φ) are indicated in bold. In addition, positively charged residues that could function as so-called Sec-avoidance signal (54) are indicated in bold and italics. The hydrophobic H-domain is indicated in gray shading. In signal peptides with a predicted signal peptidase I cleavage site, residues from position -3 to -1 relative to the signal peptidase I cleavage site are underlined. Notably, some of these proteins contain one or more putative transmembrane segments elsewhere in the protein (indicated with "TM"), or are putative lipoproteins. Residues forming a so-called lipobox for signal peptidase II cleavage are enlarged in size.

Please replace Table IV and following text, on page 59, with the following:

**Table IV.** Twin-Arginine Signal Peptides of PhoD and PhoD-like proteins\*

protein	signal peptide
PhoD (Bsu)	MAYDSRFDEWVQKLKEESFQNNRFD <b>RRKFI</b> QGAGK <b>IAGLSLGLT</b> IAQSVGA <b>FEV</b> ( <u>SEQ ID NO:52</u> )
SP1 (Sco)	MTPANHQAPTSAPSPAPSQSSHAPELRAAARSLG <b>RRRFL</b> TVTGAAAALAFAVNLPAAGTA
SP2 (Sco)	MAPTGRPSALAEHAFSPHDAVLGAAARHLG <b>RRRFL</b> TVTAAAAALAFSTNLPA
SP3 (Sco)	RGAVAAPE ( <u>SEQ ID NO:54</u> )
SP4 (Ste)	MTSRHRASENSRTPSRRT <b>VVKAAA</b> AGAVLAAPLAAALPAGAADAAPA ( <u>SEQ ID NO:55</u> )
	MTPAARPSQHAP <b>ELRAAARHLGRRRFL</b> TVTGAAAALAFAVNLPAAGT <b>AAAAEL</b> ( <u>SEQ ID NO:56</u> )

\*Homologues of *B. subtilis* PhoD were identified by amino acid sequence similarity searches in GenBank using the Blast algorithm. SP1 (Sco), gene Scc75A.32c of *Streptomyces coelicolor* (CAB61732); SP2 (Sco), gene SCF43A.18 of *S. coelicolor* (CAB48905); SP3 (Sco), gene SC4G6.37 of *S. coelicolor* (CAB51460), and SP4, phoD gene of *Streptomyces tendae* (CAB62565). GenBank Accession numbers are indicated in parenthesis. Conserved residues of the twin-arginine consensus are indicated in bold. The hydrophobic H-region is indicated by boxed text. Signal peptidase I recognition sequences predicted with the SignalP algorithm (61, 62) are underlined.